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Baicalin Improves the IL-6-Mediated Hepatic Insulin Resistance in Hepa-1c1c7 Cells

Byeong Suk Chae[1],* and Chanho Oh[2]

[1]College of Pharmacy, Woosuk University, Wanju, Jeonbuk 565-701, Republic of Korea
[2]Department of Food & Biotechnology, Woosuk University, Wanju, Jeonbuk 565-701, Republic of Korea

Abstract – Baicalin has antioxidant, anti-inflammatory and anti-diabetic properties. IL-6 is a primary proinflammatory cytokine that contributes to impaired insulin signaling in liver. This study was carried out to investigate whether baicalin improves IL-6-mediated insulin resistance in liver. Hepa-1c1c7 cells were pre-treated with 50 and 100 μM baicalin in complete media for 1 h and then cultured in the presence or absence of IL-6 (20 ng/ml). These results demonstrated that baicalin restored IL-6-suppressed expression of insulin receptor substrate (IRS)-1 protein, downregulated IL-6-increased gene expression of C-reactive protein (CRP) and suppressor of cytokine signaling (SOCS)-3, and inhibited LPS-induced production of IL-6 in Hepa-1c1c7 cells. These findings indicate that baicalin may ameliorate hepatic insulin resistance via improvement of IL-6-mediated impaired insulin signaling in hepatocytes.
Keywords – Baicalin, IL-6, Hepatic insulin resistance, SOCS-3, CRP, IRS-1

Introduction

Hepatic insulin resistance leads to the development of the metabolic syndrome and promotes progression to cardiovascular disease (Meshkani and Adeli, 2009). Hepatic insulin resistance is characterized by the impairment of insulin signaling related to suppression of glucose production in liver, which contributes to hyperglycemia and plays the key role in the whole body insulin resistance (Michael *et al*., 2000; Haas and Biddinger, 2009). Obesity is characterized by chronic low-grade inflammation and insulin resistance that can lead to type 2 diabetes and metabolic syndrome (Xu *et al*., 2003). Accumulating evidences reveal that production of adipokines in fat tissue is closely related to nonalcoholic hepatic steatosis and hepatic insulin resistance (Petersen *et al*., 2005; Li *et al*., 2013). Especially, IL-6 has emerged as a potential mediator that links obesity-derived chronic low-grade inflammation with hepatic insulin resistance (Klover *et al*., 2003; Klover *et al*., 2005).

IL-6 is a primary proinflammatory cytokine that contributes to impairment of insulin signaling in liver. IL-6 impairs insulin action and insulin signaling transduction in liver. Insulin-dependent tyrosine phosphorylation of hepatic insulin receptor substrate (IRS)-1 and IRS-2 was shown to be inhibited in chronic IL-6-treated animals (Senn *et al*., 2002). IL-6-induced gene expression of suppressor of cytokine signaling (SOCS)-3 in hepatocytes is associated with inhibition of hepatic insulin-dependent receptor autophosphorylation and IRS-1 tyrosine phosphorylation (Senn *et al*., 2003; Ueki *et al.,* 2004). IL-6 is also a strong inducer of C-reactive protein (CRP), which mediates development of hepatic insulin resistance and type 2 diabetes (Pradhan *et al*., 2001; Xi *et al*., 2011). Therefore, hepatic insulin resistance in obesity is thought to be contributed by IL-6.

Baicalin, one of the major flavonoids in *Scutellaria baicalensis*, is known to possess antioxidant and anti-inflammatory effects (Krakauer *et al*., 2001; Lixuan *et al*., 2010). Baicalin has been shown to have anti-diabetic and hypoglycemic effects through increasing the hepatic glycogen content and glycolysis and reducing the serum levels of TNF-α in diabetic rats (Li *et al*., 2011). Long-term baicalin administration reduced visceral fat mass and levels of serum TNF-α and ameliorated metabolic disorders and hepatic steatosis in rats given a high-fat diet (Guo *et al*., 2009). Also, baicalin has been reported to decrease lipid accumulation following the addition of high glucose in HepG2 cells (Guo *et al*., 2009). Therefore, we postulated that baicalin might improve

*Author for correspondence
Byeong Suk Chae, College of Pharmacy, Woosuk University, Wanju, Jeonbuk, 565-701, Republic of Korea
Tel: +82-63-290-1426; E-mail: cbse@woosuk.ac.kr

hepatic insulin resistance in obesity. However, whether baicalin improves IL-6-mediated hepatic insulin resistance remains unclear. Accordingly, the present study has been investigated effect of baicalin on the IL-6-mediated impairment of hepatic insulin signaling in Hepa-1c1c7 cells.

Experimental

Materials – Hepa-1c1c7 cells were purchased from the Korean Cell Bank (Seoul, Korea). Anti-IRS-1 and anti-β-actin antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Insulin and recombinant IL-6 and IL-1β were purchased from Sigma (St. Louis, MO, USA) except where indicated differently. Baicalin was obtained from Dae Keun Kim professor in college of pharmacy, Woosuk University and dissolved in dimethyl sulfoxide (DMSO) for *in vitro* experiment.

Cell culture – Hepa-1c1c7 cells were maintained in complete DMEM (Invitrogen, Carlsbad, CA) supplemented with 10% FBS (Sigma) and 1× antibiotic/ presence or absence of LPS 1 μg/ml (Sigma Chemical Co., St., Louse, MO) at 37 ºC in a antimycotic (Invitrogen). The cells were pretreated with various concentrations of baicalin in complete media for 1 h and then cultured for 24 h for IL-6 production in the prhumidified atmosphere containing 5% CO_2. The cell supernatants were stored at −70 ºC for cytokine assay.

Cytokine assay – The concentrations of cytokine in the supernatants from Hepa-1c1c7 cells were determined by using cytokine monoclonal antibodies (BD Biosciences Pharmingen, U.S.A.). All measurements were carried out in triplicate. The results were measured in picograms per milliliter at 450 nm using an ELISA microplate reader (Molecular Devices Co., Ltd., U.S.A.). The lower limit of sensitivity for each of the ELISA was equal to or smaller than 5 pg/ml.

Western blot analysis – Following preincubation with baicalin and treatment of IL-6 or insulin, Hepa-1c1c7 cells were washed with ice-cold PBS and then scraped from the plate in 500 μl of lysis buffer (20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM Na_2-EDTA, 1 mM EGTA, 1% Triton, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na_3VO_4, 1 μg/ml leupeptin, and 1 mM PMSF). After 30 min at 4 ºC, the lysates were centrifuged (15,000 × g for 15 min) and stored at −80 ºC until use. Proteins were separated on SDS-PAGE and transferred to polyvinylidene fluoride (PVDF). Nonspecific binding was blocked with TBS-T (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.1% Tween 20) containing 5% non-fat milk for 1 h. Primary antibodies were diluted and incubated with membranes overnight at 4 ºC with agitation. After washing three times with TBS-T, secondary antibodies were incubated for 1 h. After 5 additional washes with TBS-T, the bands were visualized with chemiluminescence according to the manufacturer's instructions.

Total RNA isolation and RT-PCR – Following preincubation with baicalin for 1 h, Hepa-1c1c7 cells were incubated for indicated hours in the presence or absence of IL-6 20 ng/ml or IL-1β 1 ng/ml at 37 ºC, and 5% CO_2. Total RNA was extracted from the cells using an RNA purification kit (QIAGEN) according to the manufacturer's instructions and quantitated spectrophotometrically at 260 nm. cDNA synthesis from total RNA (2 μg) was performed with QuantiTect® Reverse Transcription kit (QIAGEN). PCR was performed in a 20 μl final volume containing 2 μl of the first strand cDNA, 1 μM of sense and antisense primers (BIONEER, Kor.), and 10 μl of 400 nM of QuantiTect® SYBR Green PCR Master Mix (QIAGEN) using a MultiGene PCR (Labnet International Inc.). With a housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), as an internal control, was amplified by PCR at the same time. Amplification was performed for 15 min at 95 ºC, followed by 40 cycles of denaturation at 95 ºC for 15 s, annealing at 55 ºC for 30 s, and extension at 72 ºC for 30 s in a thermocycler (GeneAmp 9600-R, Perkin-Elmer, Wellesley, MA). The primers were as follows: SOCS-3 (sense 5'-CAGCTCCAAAAGCGAGTACCA-3' and antisense 5'-CGGTCACGGGCGCTCCAGTAGA-3'); CRP (sense 5'-AGATTCCTGCTCCAACAC-3' and antisense 5'-TCAA AGTCACCGCCATACGA-3'); GAPDH (sense 5'-GCC AAGGTCATCCATGACAAC-3' and antisense 5'-AGT GTAGCCCAAGATGCCCTT-3') was used as positive control. The amplified PCR products were analyzed by electrophoresis on a 1.2% agarose gels and visualized by ethidium bromide staining. Quantification of the band intensity on the Hyperfilm was performed using the public domain NIH image software.

Statistical Analysis – All data were expressed as means ± standard error (S.E.). Experiments were always run in triplicate and repeated at least twice. Analysis of variation and Student's *t*-test were used to determine statistical significance, and $p < 0.05$ was considered to be statistically significant.

Results and Discussion

Baicalin increased IL-6-suppressed expression of IRS-1 in Hepa-1c1c7 cells – It has been reported that

baicalin possesses antihyperglycemic effects (Li, *et al*., 2011), reduces visceral fat mass, and ameliorates metabolic disorders and hepatic steatosis in rats given a high- fat diet (Guo *et al*., 2009). However, whether baicalin improves hepatic insulin resistance in obesity remains unclear. Adipose tissue contributed to 10–35% of circulating IL-6 in resting, healthy humans (Mohamed-Ali *et al*., 1997), and the production of IL-6 was greater by visceral adipose tissue than other adipose tissue (Fain *et al*., 2004). IL-6 is a potential mediator that links obesity-derived chronic low-grade inflammation with hepatic insulin resistance. IL-6 depletion selectively improved hepatic insulin action in obesity (Klover *et al*., 2005). Chronic exposure to IL-6 caused hepatic insulin resistance in mice (Klover *et al*., 2003). IL-6 induces abnormalities in expression and secretion of numerous proteins influencing the signal transduction pathways of insulin receptor at the cellular level in hepatocytes, which may result in incomplete suppression of hepatic glucose production (Senn *et al*., 2002). Hepatic IRS-1 and IRS-2 have complementary roles in the control of hepatic metabolism including glucose homeostasis and lipid metabolism (Tanti *et al*., 1994). Downregulated expression of IRS-1 in liver is associated with inhibition of insulin receptor tyrosine kinase activity in obesity-induced insulin resistance (Hotamisligil *et al*., 1996). Insulin-dependent tyrosine phosphorylation of hepatic IRS-1 and IRS-2 was inhibited in chronic IL-6-treated animals (Senn *et al*., 2002). We examined to investigate whether baicalin reverses IL-6-mediated inhibition of IRS-1 expression in liver. Guo *et al*. (2009) has reported that baicalin at concentration of 50 and 100 μM decreased lipid accumulation following the addition of high glucose in HepG2 cells. In addition, our previous study was observed that 50 and 100 μM baicalin remarkably inhibited TNF-α-induced production of IL-6 by differentiated 3T3-L1 cells in a dose-dependent manner (data not shown). Accordingly, in the present study we used 50 and 100 μM baicalin in Hepa-1c1c7 cells. Hepa-1c1c7 cells were pretreated with 50 and 100 μM baicalin in complete media for 1 h and cultured in the presence or absence of IL-6 20 ng/ml at 37 ºC, 5% CO_2 incubation for 2 h. And then the cells were stimulated with 100 nM human insulin for last 3 min at 37 ºC. The cells were harvested and total proteins were isolated from the cells for western blot analysis. As indicated in Fig. 1, the results demonstrated that baicalin reversed IL-6-supressed expression of IRS-1 protein in the presence of insulin in Hepa-1c1c7 cells in a concentration-dependent manner. Therefore, these results suggest that baicalin may ameliorate hepatic insulin resistance through upregulation of IL-6-suppressed expression of IRS-1 in hepatocytes.

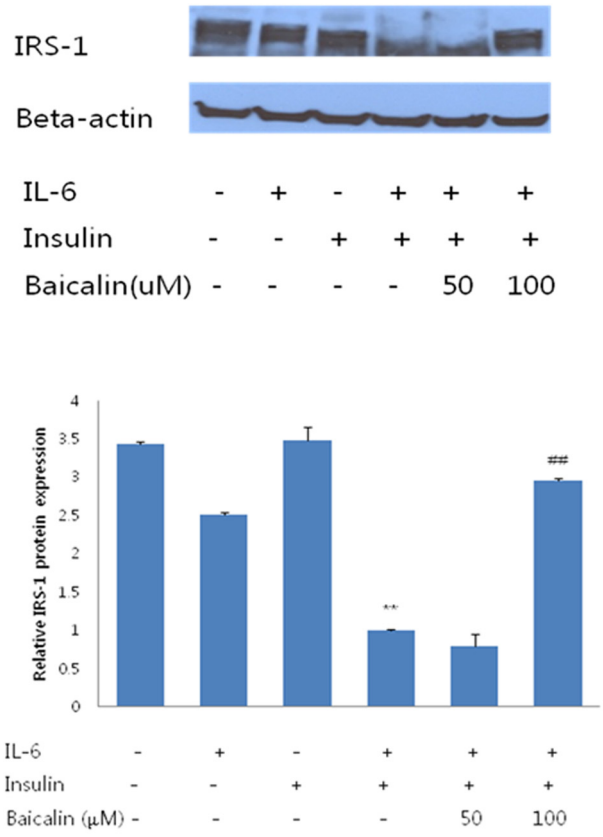

Fig. 1. Baicalin upregulates the IL-6-suppressed expression of IRS-1 protein in Hepa-1c1c7 cells - Hepa-1c1c7 cells were pretreated with 50 and 100 μM baicalin for 1 h and then cultured for 2 h in the presence or absence of IL-6 (20 ng/ml). Thereafter, the cells were stimulated with 100 nM human insulin for 3 min at 37 ºC. Protein expression was determined by western blot. $^{**}(p < 0.01)$: Significantly different from the value in negative control. $^{\#\#}(p < 0.01)$: Significantly different from the value in positive controls.

Baicalin attenuated IL-6-induced gene expression of SOCS-3 in Hepa-1c1c7 cells – SOCS-3 is an insulin-induced negative regulator of insulin signaling and a potent mediator of IL-6-dependent insulin resistance in liver (Senn *et al.,* 2003). IL-6-induced gene expression of hepatic SOCS-3 is associated with inhibition of hepatic insulin-dependent receptor autophosphorylation and IRS-1 tyrosine phosphorylation (Ueki *et al.,* 2004). In this study, to investigate whether baicalin attenuates IL-6-mediated gene expression of SOCS-3 in liver, Hepa-1c1c7 cells were pre-treated with 50 and 100 μM baicalin in complete media for 1 h and then cultured for 1 h in the presence or absence of IL-6 20 ng/ml at 37 ºC, 5% CO_2 incubation. The cells were harvested and total RNA was isolated, and the presence of SOCS-3 mRNA was examined by RT-PCR analysis. Our results demonstrated

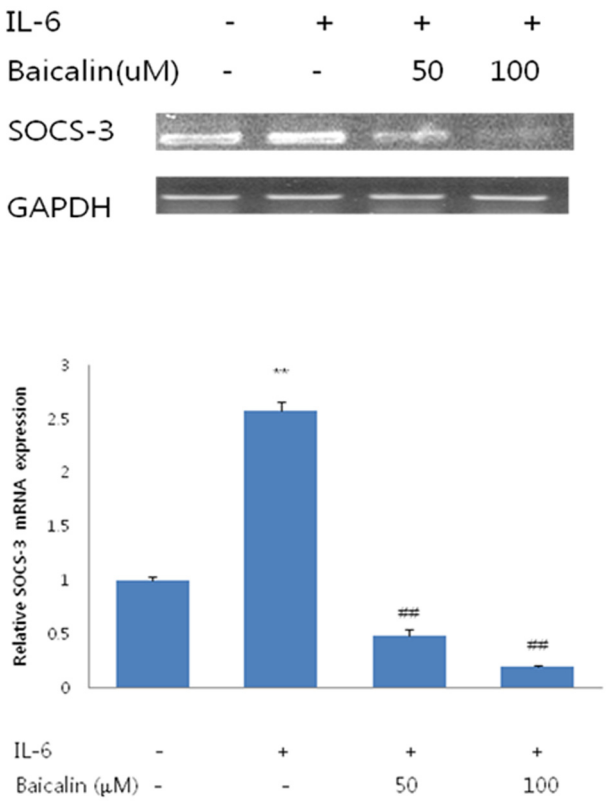

Fig. 2. Baicalin suppresses the IL-6-induced expression of SOCS3 mRNA in Hepa-1c1c7 cells - Hepa-1c1c7 cells were pretreated with baicalin and then cultured for 1 h for SOCS-3 mRNA in the presence or absence of IL-6. Total RNA was then isolated from the cells and gene expression was determined by RT-PCR. Other legends and methods are the same as in Fig. 1. $^{**}(p < 0.01)$: Significantly different from the value in negative control. $^{\#\#}(p < 0.01)$: Significantly different from the value in positive controls.

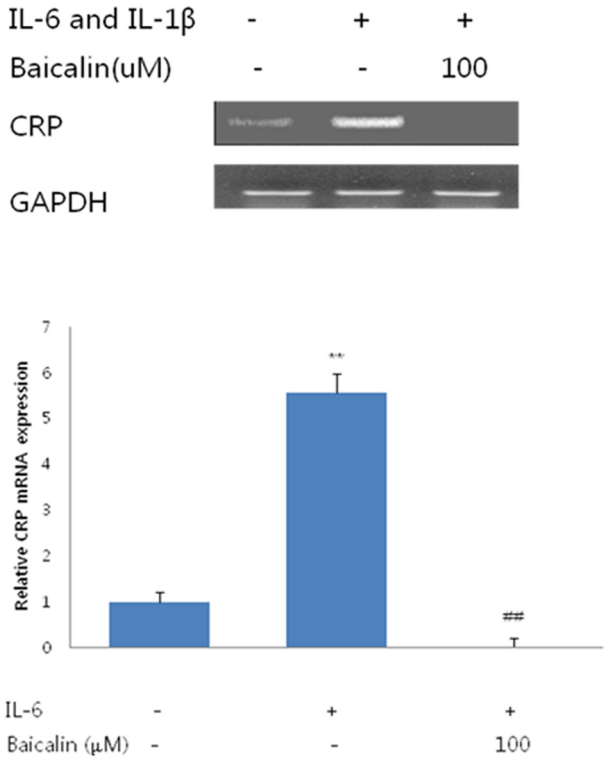

Fig. 3. Baicalin attenuates CRP mRNA expression induced by IL-6 and IL-1β in Hepa-1c1c7 cells - Hepa-1c1c7 cells were pretreated with baicalin for 1 h and then cultured for 18 h in the presence or absence of IL-6 and IL-1β for CRP mRNA expression. Other legends and methods are the same as in Fig. 2. $^{**}(p < 0.01)$: Significantly different from the value in negative control. $^{\#\#}(p < 0.01)$: Significantly different from the value in positive controls.

that baicalin significantly attenuated IL-6-induced gene expression of SOCS-3 in Hepa-1c1c7 cells in a concentration-dependent manner (Fig. 2), suggesting that baicalin may ameliorate impaired insulin sensitivity in liver via downregulation of IL-6-induced gene expression of SOCS-3 in hepatocytes.

Baicalin suppressed gene expression of CRP induced by IL-6 and IL-1β in Hepa-1c1c7 cells – CRP, an acute phase protein, is well-known as a potent predictor of future metabolic syndrome and cardiovascular disease (Ridker and Morrow, 2003). CRP has been reported to mediate development of hepatic insulin resistance and type 2 diabetes (Pradhan *et al*., 2001; Xi *et al*., 2011). CRP expression has been shown to be mainly mediated by the transcription factor STAT3 (Zhang *et al.,* 1996), which can lead to development of hepatic insulin resistance and type 2 diabetes (Pradhan *et al.,* 2001). Xi *et al*. (2011) has reported that CRP induces impairment of insulin signaling via enhanced phosphorylation of MAPK/extracellular signal-regulated kinase (ERK)1/2 and p38 in primary cultured rat hepatocytes. IL-6 is well-known as a strong inducer of CRP production in liver. IL-1β alone could not induce CRP in hepatoma cell lines, while IL-6 alone or both of IL-6 and IL-1β were considered as strong inducers of CRP (Ganapathi *et al*., 1991; Kramer *et al*. 2008). In the present study, we used both of IL-6 20 ng/ml and IL-1β 1 ng/ml to strongly induce CRP mRNA in Hepa-1c1c7 cells. In the present study, Hepa-1c1c7 cells were pre-treated with 100 μM baicalin in complete media for 1 h and then cultured for 18 h in the presence or absence of IL-6 20 ng/ml and IL-1β 1 ng/ml at 37 °C in a humidified atmosphere containing 5% CO_2. The cells were harvested and total RNA was then isolated from the cells, and then CRP gene expression was examined using RT-PCR. As shown in Fig. 3, the results demonstrated that baicalin attenuated increased gene expression of CRP by treatment of both IL-6 and IL-1β in Hepa-1c1c7 cells (Fig. 3). Therefore, these results indicate that baicalin may

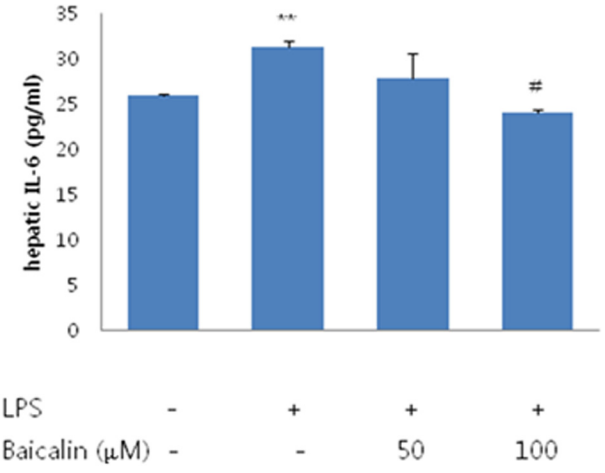

Fig. 4. Baicalin inhibits the LPS-induced production of IL-6 in Hepa-1c1c7 cells - Hepa-1c1c7 cells were preincubated with baicalin for 1 h and then cultured for 24 h in the presence or absence of LPS. Concentrations of cytokine were measured using ELISA. Each value represents the mean ± S.E. $^{**}(p < 0.01)$: Significantly different from the value in negative control. $^{\#}(p < 0.05)$: Significantly different from the value in positive controls.

improve insulin resistance in liver through downregulation of IL-6-induced gene expression of CRP in hepatocytes.

Baicalin inhibited LPS-induced production of IL-6 in Hepa-1c1c7 cells – LPS induces hepatocyte injury and increases expression of TNF-α and IL-6 in hepatocytes (Zhou *et al.,* 2012). LPS-induced production of IL-6 by hepatocytes contributes to acute-phase response in liver (Saad *et al*., 1995), indicating that enhanced IL-6 in hepatocytes may contribute, in part, to the impaired hepatic insulin sensitivity via induction of CRP in hepatocytes. Therefore, hepatic IL-6 production may be a target for improvement of insulin resistance in hepatocytes. Many studies demonstrated that baicalin had anti-inflammatory, anti-bacterial and antiviral activities (Krakauer *et al*., 2001; Lixuan *et al*., 2010). Our previous data demonstrated that baicalin inhibited production and gene expression of IL-6 by differentiated 3T3-L1 adipocytes (data not shown). In the present study, to further investigate whether baicalin inhibits LPS-induced production of IL-6 in Hepa-1c1c7 cells, the cells were pretreated with 50 and 100 μM baicalin in complete media for 1 h and then cultured for 24 h in the presence or absence of LPS 1 μg/ml at 37 °C in a humidified atmosphere containing 5% CO_2. These results demonstrated that 100 μM baicalin significantly inhibited LPS-induced production of IL-6 (Fig. 4). As shown in Fig. 3, 100 μM baicalin attenuated IL-6-induced gene expression of CRP in Hepa-1c1c7 cells. Therefore, our observation suggests that baicalin may improve IL-6-induced impaired insulin signaling via inhibition of production of hepatic IL-6 and consequently CRP.

In conclusion, these findings indicate that baicalin may improve impaired hepatic insulin signaling via upregulation of IL-6-suppressed expression of IRS-1, downregulation of IL-6-induced gene expression of SOCS-3 and CRP, and inhibition of production of IL-6 in hepatocytes.

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References

Emanuelli, B., Peraldi, P., Filloux, C., Sawka-Verhelle, D., Hilton, D., and Van Obberghen, E., SOCS-3 is an insulin-induced negative regulator of insulin signaling. *J. Biol. Chem*. **275**, 15985-15991 (2000).

Fain, J.N., Madan, A.K., Hiler, M.L., Cheema, P., and Bahouth, S.W., Comparison of the release of adipokines by adipose tissue, adipose tissue matrix, and adipocytes from visceral and subcutaneous abdominal adipose tissues of obese humans. *Endocrinology* **145**, 2273-2282 (2004).

Guo, H.X., Liu, D.H., Ma, Y., Liu, J.F., Wang, Y., Du, Z.Y., Wang, X., Shen, J.K., and Peng, H.L., Long-term baicalin administration ameliorates metabolic disorders and hepatic steatosis in rats given a high-fat diet. *Acta. Pharmacol. Sin*. **30**, 1505-1512 (2009).

Ganapathi, M.K., Rzewnicki, D., Samols, D., Jiang, S.L., and Kushner, I., Effect of combinations of cytokines and hormones on synthesis of serum amyloid A and C-reactive protein in Hep 3B cells. *J. Immunol*. **147**, 1261-1265 (1991).

Haas, J.T. and Biddinger, S.B., Dissecting the role of insulin resistance in the metabolic syndrome. *Curr. Opin. Lipidol*. **20**, 206-210 (2009).

Hotamisligil, G.S., Peraldi, P., Budavari, A., Ellis, R., White, M.F., and Spiegelman, B.M., IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNFα and obesity-induced insulin resistance. *Science* **271**, 665-668 (1996).

Klover, P.J., Clementi, A.H., and Mooney, R.A., Interleukin-6 depletion selectively improves hepatic insulin action in obesity. *Endocrinology* **146**, 3417-3427 (2005).

Klover, P.J., Zimmers, T.A., Koniaris, L.G., and Mooney, R.A., Chronic exposure to interleukin-6 causes hepatic insulin resistance in mice. *Diabetes* **52**, 2784-2789 (2003).

Krakauer, T., Li, B.Q., and Young, H.A., The flavonoid baicalin inhibits superantigen-induced inflammatory cytokines and chemokines. *FEBS Lett*. **500**, 52-55 (2001).

Kramer, F., Torzewski, J., Kamenz, J., Veit, K., Hombach, V., Dedio, J., and Ivashchenko, Y., Interleukin-1beta stimulates acute phase response and C-reactive protein synthesis by inducing an NFkappaB- and C/EBPbeta-dependent autocrine interleukin-6 loop. *Mol. Immunol*. **45**, 2678-2689 (2008).

Li, H.T., Wu, X.D., Davey, A.K., and Wang, J., Antihyperglycemic effects of baicalin on streptozotocin - nicotinamide induced diabetic rats. *Phytother. Res*. **25**, 189-194 (2011).

Lixuan, Z., Jingcheng, D., Wenqin, Y., Jianhua, H., Baojun, L., and Xiaotao, F., Baicalin attenuates inflammation by inhibiting NF-kappaB activation in cigarette smoke induced inflammatory models.

Pulm. Pharmacol. Ther. **23**, 411-419 (2010).
Li, Y., Ding, L., Hassan, W., Abdelkader, D., and Shang, J., Adipokines and hepatic insulin resistance. *J. Diabetes Res.* **2013**, 170532-170539 (2013).
Michael, M.D., Kulkarni, R.N., Postic, C., Previs, S.F., Shulman, G.I., Magnuson, M.A., and Kahn, C.R., Loss of insulin signaling in hepatocytes leads to severe insulin resistance and progressive hepatic dysfunction. *Mol. Cell*. **6**, 87-97 (2000).
Meshkani, R. and Adeli, K., Hepatic insulin resistance, metabolic syndrome and cardiovascular disease. *Clin. Biochem*. **42**, 1331-1346 (2009).
Petersen, K.F., Dufour, S., Befroy, D., Lehrke, M., Hendler, R.E., and Shulman, G.I., Reversal of nonalcoholic hepatic steatosis, hepatic insulin resistance, and hyperglycemia by moderate weight reduction in patients with type 2 diabetes. *Diabetes* **54**, 603-608 (2005).
Pradhan, A.D., Manson, J.E., Rifai, N., Buring, J.E., and Ridker, P.M., C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *J. Am. Med. Assoc*. **286**, 327-334 (2001).
Ridker, P.M. and Morrow, D.A., C-reactive protein, inflammation, and coronary risk. *Cardiol. Clin*. **21**, 315-325 (2003).
Saad, B., Frei, K., Schol, F.A., Fontana, A., and Maier, P., Hepatocyte-derived interleukin-6 and tumor-necrosis factor alpha mediate the lipopolysaccharide-induced acute-phase response and nitric oxide release by cultured rat hepatocytes. *Eur. J. Biochem*. **229**, 349-355 (1995).
Senn, J.J., Klover, P.J., Nowak, I.A., and Mooney, R.A., Interleukin-6 induces cellular insulin resistance in hepatocytes. *Diabetes* **51**, 3391-3399 (2002).
Senn, J.J., Klover, P.J., Nowak, I.A., Zimmers, T.A., Koniaris, L.G., Furlanetto, R.W., and Mooney, R.A., Suppressor of cytokine signaling-3 (SOCS-3), a potential mediator of interleukin-6-dependent insulin resistance in hepatocytes. *J. Biol. Chem*. **278**, 13740-13746 (2003).
Tanti, J.F., Gremeaux, T., van Obberghen, E., and Le Marchand-Brustel, Y., Serine/threonine phosphorylation of insulin receptor substrate 1 modulates insulin receptor signaling. *J. Biol. Chem*. **269**, 6051-6057 (1994).
Ueki, K., Kondo, T., and Kahn, C.R., Suppressor of cytokine signaling 1 (SOCS-1) and SOCS-3 cause insulin resistance through inhibition of tyrosine phosphorylation of insulin receptor substrate proteins by discrete mechanisms. *Mol. Cell. Biol*. **24**, 5434-5446 (2004).
Xi, L., Xiao, C., Bandsma, R.H., Naples, M., Adeli, K., and Lewis, G.F., C-reactive protein impairs hepatic insulin sensitivity and insulin signaling in rats: Role of mitogen-activated protein kinases. *Hepatology* **53**, 127-135 (2011).
Xu, H., Barnes, G.T., Yang, Q., Tan, G., Yang, D., Chou, C.J., Sole, J., Nichols, A., Ross, J.S., Tartaglia, L.A., and Chen, H., Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J. Clin. Invest*. **112**, 1821-1830 (2003).
Zhang, D., Sun, M., Samols, D., and Kushner, I., STAT3 participates in transcriptional activation of the C-reactive protein gene by interleukin-6. *J. Biol. Chem*. **271**, 9503-9509 (1996).
Zhou, J., Wang, Q., Wang, Q., and Duan, W., Effects of norcantharidin on lipopolysaccharide-induced hepatocyte injury in vitro. *Zhong Nan Da Xue Xue Bao Yi Xue Ban* **37**, 285-289 (2012).

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